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Research Progress on the Effects and Mechanisms of Hirudin on the Stability of Atherosclerotic Plaques

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Abstract

Atherosclerosis is a chronic cardiovascular disease with a high incidence in the world. Plaque stability is the core key factor to regulate its progress and the occurrence of cardiovascular events. Acute thrombotic events caused by unstable plaque rupture are the primary cause of myocardial infarction and stroke, which seriously threaten human health. Hirudin, as the core active ingredient of leeches, is a natural peptide extracted from their salivary glands. It has multiple biological functions, such as anticoagulant, anti-inflammatory, and antiplatelet aggregation, and has become a focus of attention in the field of anti-AS research. This article systematically reviews relevant research results at home and abroad and finds that hirudin can not only form stable complexes by tightly binding with thrombin, specifically inhibiting thrombin activity to exert potent anticoagulant effects, but also regulate the stability of AS plaques through multi-target and multi-pathway regulation. Its potential mechanism of action includes regulating blood lipid metabolism and reducing lipid deposition in the blood vessel wall; Inhibiting inflammatory signaling pathways such as NF-κB and reducing inflammation infiltration within plaques; exerting antioxidant stress response and reducing oxidative damage to vascular endothelium; And blocking platelet aggregation-related signaling pathways to reduce the risk of thrombosis. This study aims to provide new theoretical support and potential therapeutic strategies for the precise prevention and treatment of atherosclerosis-related cardiovascular diseases by elaborating the influence of hirudin on the stability of atherosclerotic plaque and its underlying mechanism.

Keywords

Hirudin; Atherosclerosis; Plaque Stability; Mechanism

Atherosclerosis (AS) is a chronic cardiovascular disease that poses a serious threat to human health, with a high incidence and mortality rate globally [1]. According to statistics from the World Health Organization (WHO), cardiovascular diseases cause approximately 17.9 million deaths annually, accounting for 31% of global mortality. Atherosclerosis (AS) serves as the principal pathological basis underlying cardiovascular diseases [2]. The main pathological features of AS are thickening and hardening of the arterial wall, accompanied by the formation of atherosclerotic plaques within the lumen, leading to vascular stenosis and impaired blood flow. These plaques are composed of lipids, smooth muscle cells, macrophages, and extracellular matrix, among other components. The stability of the plaques plays a crucial role in the onset and progression of cardiovascular events [3]. Hirudin is a natural polypeptide extracted from the salivary glands of leeches, possessing potent anticoagulant activity. It can bind tightly with thrombin (in a 1:1 ratio), forming a stable complex that inhibits thrombin activity and prevents progression of the coagulation process [4]. In recent years, an increasing number of studies have indicated [5-9]. Hirudin, in addition to its anticoagulant properties, also plays significant roles in lipid regulation, anti-inflammation, antioxidation, and inhibition of platelet aggregation. These mechanisms are closely associated with the maintenance of atherosclerotic plaque stability in AS. Research into the effects of hirudin on plaque stability in AS will provide new perspectives and approaches for the prevention and treatment of AS and is of considerable theoretical and clinical significance.

1. Overview of the Stability of Atherosclerotic Plaques

1.1 Mechanisms of Atherosclerosis Formation

The pathogenesis of atherosclerosis involves multiple interacting factors, with core mechanisms including lipid deposition, inflammatory responses, and endothelial injury. When lipid metabolism is abnormal, low-density lipoprotein (LDL) penetrates the subendothelial space and becomes oxidized to ox-LDL (oxidized LDL), which induces the differentiation of monocytes into macrophages. After phagocytosing ox-LDL, these macrophages form foam cells, which then accumulate to form a lipid core, laying the foundation for plaque formation [10]. Inflammatory responses persist throughout the entire course of AS (atherosclerosis). Following vascular endothelial injury, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are released, attracting the aggregation of leukocytes and monocytes. After monocytes differentiate into macrophages, they phagocytose oxidized low-density lipoprotein (ox-LDL) and form foam cells, while simultaneously secreting more inflammatory mediators, thereby exacerbating the inflammatory response. The proteases released by these cells further degrade the structure of the vascular wall, contributing to plaque instability [11]. Endothelial injury, as the initiating event, is induced by risk factors such as hypertension and hyperglycemia, leading to increased vascular permeability, decreased secretion of nitric oxide (NO), and elevated levels of endothelin-1 (ET-1), ultimately promoting thrombosis and vasoconstriction [12].

1.2 Factors Affecting Plaque Stability

Plaque stability is determined by a combination of fibrous cap thickness, lipid core size, and degree of inflammatory cell infiltration. The fibrous cap is mainly composed of smooth muscle cells and extracellular matrix, with its thickness positively correlated with mechanical strength. When inflammatory cell infiltration occurs, the secretion of matrix metalloproteinases (MMPs) increases, leading to extracellular matrix degradation and making the fibrous cap thinner and weaker, thereby increasing the risk of plaque rupture. Notably, the risk of plaque rupture increases significantly when fibrous cap thickness is less than 65 μm [13]. The lipid core is the main component of atherosclerotic plaques, and its size is closely related to plaque stability. A large lipid core, which is rich in cholesterol crystals and necrotic cellular debris, increases the volume of the plaque and exerts greater pressure on the fibrous cap, attracting inflammatory cell infiltration and disrupting the structural integrity of the cap. Oxidized low-density lipoprotein (ox-LDL) within the lipid core can activate inflammatory signaling pathways, promote inflammatory responses, and reduce plaque stability [14]. Studies have shown [15]. When the lipid core comprises more than 40% of the plaque area, the plaque is more prone to rupture. Infiltration of inflammatory cells is one of the key factors affecting plaque stability. Inflammatory cells such as macrophages and T lymphocytes accumulate within the plaque and secrete inflammatory mediators and cytokines, including TNF-α, interleukin-6 (IL-6), and interferon-γ (IFN-γ). These substances activate the expression of matrix metalloproteinases (MMPs), which degrade the extracellular matrix of the fibrous cap, resulting in the thinning of the fibrous cap. Inflammatory cells can also release reactive oxygen species (ROS), thereby promoting lipid oxidation and perpetuating a vicious cycle of inflammation, which further impairs plaque stability. Studies have shown that the greater the degree of inflammatory cell infiltration within the plaque, the higher the risk of plaque rupture.

2. Biological Characteristics of Hirudin

2.1 Structure and Function of Hirudin

Hirudin is a naturally occurring anticoagulant protein extracted from the salivary glands of leeches, characterized by a unique structure and high specificity. Hirudin consists of a single-chain polypeptide composed of 65-66 amino acid residues, containing three disulfide bonds within its molecular structure, which form a distinct spatial conformation. The principal function of hirudin is to specifically inhibit thrombin (coagulation factor IIa, Thrombin) activity. It binds tightly to both the catalytic site and substrate recognition site of thrombin, thereby blocking the coagulating action of thrombin and exerting anticoagulant effects. In addition, hirudin possesses other potential biological activities, such as inhibiting platelet aggregation and modulating cytokine expression; these functions may be related to its influence on atherosclerotic plaque stability.

2.2 Mechanisms of Action of Hirudin

The anticoagulant mechanism of hirudin is mainly based on its high affinity and specificity for thrombin inhibition. Thrombin plays a central role in the coagulation cascade by catalyzing the conversion of fibrinogen to fibrin and promoting thrombus formation. By binding to thrombin, hirudin effectively inhibits its activity, prevents the formation of fibrin clots, and accordingly exhibits anticoagulant effects, as illustrated in Figure 1. Compared to traditional anticoagulants such as heparin, hirudin provides a more direct and specific anticoagulant action. Heparin requires antithrombin III (Antithrombin III) as a cofactor, whereas hirudin binds to thrombin directly and does not depend on endogenous auxiliary factors. Furthermore, the anticoagulant effect of hirudin is more predictable and controllable, with a relatively lower risk of bleeding. These advantages confer significant clinical value for hirudin in anticoagulant therapy. Beyond its anticoagulant function, hirudin can also regulate various signaling pathways, influencing processes such as cell proliferation, differentiation, and inflammatory responses. Studies have shown Hirudin may affect atherosclerotic plaque stability by inhibiting the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, thereby reducing the expression of inflammatory cytokines and inhibiting the proliferation of vascular smooth muscle cells (VSMCs).

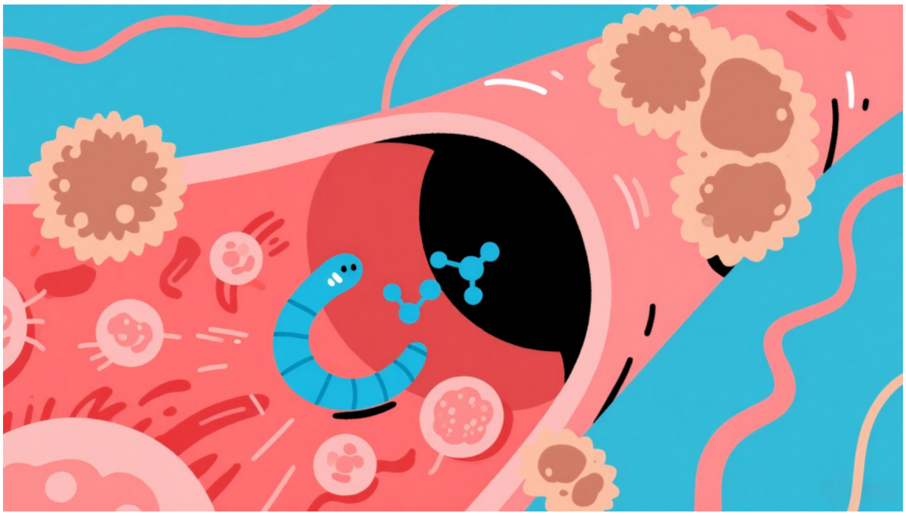

Figure 1. Schematic diagram of the mechanism of specific binding of hirudin and thrombin to inhibit coagulation.

3. Studies on the Effect of Hirudin on the Stability of Atherosclerotic Plaques

3.1 Animal Experimental Studies

Animal experiments have extensively demonstrated the positive impact of hirudin on the stability of atherosclerotic (AS) plaques. Tian et al. utilized a high-fat diet-induced AS mouse model and found that after 12 weeks of intraperitoneal hirudin administration, the area of aortic plaques and lipid deposition were significantly reduced. Oil Red O staining showed decreased lipid content within plaques and a reduction in the number of foam cells, indicating that hirudin can inhibit lipid deposition and foam cell formation, shrink the lipid core of plaques, and enhance plaque stability. Inflammatory responses are key factors leading to rupture of atherosclerotic plaques. In a study using a rabbit AS model, after 8 weeks of intragastric administration of hirudin, the infiltration of inflammatory cells within the plaques, specifically macrophages and T lymphocytes, was reduced, and the expression levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), were significantly decreased. Mechanistic studies have demonstrated that hirudin may reduce the disruption of plaque stability caused by inflammation by inhibiting the activation of the NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway, thereby decreasing the transcription and expression of inflammatory cytokines, ameliorating inflammation-induced damage to the fibrous cap, maintaining its integrity, and lowering the risk of plaque rupture. In addition, hirudin also affects intraplaque neovascularization. In atherosclerosis, increased neovascularization within plaques can lead to intraplaque hemorrhage and exacerbated inflammation, further reducing plaque stability. According to Pang et al. Studies have found that in rat models of diabetic nephropathy, hirudin significantly reduces the expression of hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor

(VEGF). Yan Yuyong et al. Observations using Micro-CT and three-dimensional reconstruction techniques demonstrate that native hirudin can significantly improve the survival rate of ischemic flaps in rats and promote angiogenesis within the flap tissue. Peng et al. the research further revealed that natural hirudin significantly increased flap survival rates by activating the protease-activated receptor (PARs)/p38/NF-κB signaling pathway.

3.2 Clinical Research

The efficacy and safety of hirudin in the treatment of atherosclerosis-related diseases have attracted considerable attention. Multiple studies have demonstrated its efficacy in improving patient conditions and enhancing plaque stability. Liu Xuhong et al. Studies have found that, on the basis of conventional treatment for acute cerebral infarction, the addition of Mai Xue Kang capsules (the main active ingredient being hirudin) leads to a significant reduction in serum endothelin-1 (ET-1) and thromboxane B_2 (TXB_2) levels, while levels of nitric oxide (NO) increase markedly. ET-1, a potent vasoconstrictive factor, when decreased, contributes to vasodilation; NO, as a major vasodilatory factor, can inhibit platelet aggregation and inflammatory responses. These findings suggest that hirudin may improve vascular endothelial function and indirectly enhance the stability of atherosclerotic plaques, offering new insights for the treatment of cerebral infarction. Lin Guanyu et al.'s studies have shown that low (1 ATU/mL) and moderate (4 ATU/mL) concentrations of native hirudin significantly promote the proliferation of human microvascular endothelial cells (HMVECs) and microvessel formation, whereas high concentrations (7 ATU/mL) exert an inhibitory effect. Yang Baogang et al. Studies have found that Leech Capsule can reduce the levels of ET-1 (Endothelin-1) and TXB_2 (Thromboxane B_2), while increasing the levels of NO (Nitric Oxide) and 6-Keto-PGF1α (6-Keto-Prostaglandin F1α). This significantly improves vascular endothelial function, helps maintain the integrity of the vascular wall, and reduces the adhesion and migration of inflammatory cells, thereby stabilizing atherosclerotic plaques. This may represent an important mechanism by which Leech Capsule reduces major adverse cardiovascular events (MACE) in patients following coronary stenting. In addition, other studies have found that after administration of hirudin-containing medications in patients with coronary artery disease, lipid profiles improved and inflammatory markers such as C-reactive protein (CRP) decreased. Dyslipidemia and inflammatory responses are important factors affecting plaque stability, and hirudin may positively impact plaque stability by regulating lipid levels and inhibiting inflammation.

4. Discussion on the Mechanisms of Hirudin Influencing Atherosclerotic Plaque Stability

4.1 Regulation of Lipid Metabolism

Dysregulation of lipid metabolism is a major risk factor for the development and progression of atherosclerosis, and hirudin has demonstrated a beneficial role in modulating lipid metabolism, thereby influencing the stability of atherosclerotic plaques. Multiple studies have indicated that hirudin can reduce blood levels of total cholesterol (TC) and triglycerides (TG). Yang Hongyan et al. found that hirudin could regulate lipid metabolism in rabbits with blood stasis syndrome, lowering serum levels of TC, TG, and low-density lipoprotein cholesterol (LDL-C), while upregulating the activity of antioxidant enzyme superoxide dismutase (SOD) and modulating the expression of genes related to lipid metabolism. Further studies have revealed that hirudin has been shown to upregulate the expression of the apolipoprotein A-I (ApoA-I) gene in the liver. ApoA-I is the principal apolipoprotein of high-density lipoprotein (HDL), and it promotes reverse cholesterol transport by facilitating the transfer of cholesterol from peripheral tissues back to the liver for metabolism. This process helps lower blood cholesterol levels and reduces lipid deposition in the vascular wall. In addition, hirudin can downregulate the expression of the apolipoprotein B (ApoB) gene, which encodes the main apolipoprotein of low-density lipoprotein (LDL). Reduced ApoB expression contributes to decreased LDL production, thereby lowering the plasma levels of LDL cholesterol (LDL-C). By modulating lipid metabolism, hirudin reduces lipid deposition in the vascular wall, decreases the volume of the lipid core within the plaque, thereby increasing the stability of atherosclerotic plaques.

4.2 Inhibition of Inflammatory Responses

The inflammatory response plays a critical role in atherosclerosis. Hirudin can suppress the inflammatory response through multiple pathways, thereby protecting vascular endothelial function and stabilizing atherosclerotic plaques. Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) serve as key mediators in atherosclerosis, and hirudin has been shown to significantly inhibit the release of these cytokines. In cell culture experiments, after stimulation of macrophages with lipopolysaccharide (LPS) to induce an inflammatory response, the addition of hirudin resulted in a significant reduction in the levels of TNF-α (tumor necrosis factor-alpha), IL-1 (interleukin-1), and IL-6 (interleukin-6) in the cell culture supernatant. This indicates that hirudin is capable of inhibiting the activation of inflammatory cells and reducing the production of inflammatory mediators, thereby mitigating damage to the vascular wall. Modulation of inflammatory signaling pathways is an important mechanism by which hirudin exerts its anti-inflammatory effects. Studies have shown that hirudin can inhibit the activation of the nuclear factor-kappa B (NF-κB) signaling pathway. NF-κB, a pivotal transcription factor, upon activation, translocates to the cell nucleus to regulate the expression of inflammation-related genes. Hirudin may inhibit the activity of IκB kinase (IKK), preventing IκB phosphorylation and degradation, thus keeping NF-κB in an inactive state and unable to initiate transcription of proinflammatory genes, resulting in suppression of inflammatory cytokine expression.

4.3 Anti-Oxidative Stress Effects

Oxidative stress plays a crucial role in atherosclerosis, leading to vascular endothelial cell injury, lipid peroxidation, and exacerbation of inflammatory responses, which, in turn, affect plaque stability. Hirudin possesses significant antioxidative stress properties, capable of scavenging free radicals, alleviating oxidative damage, and stabilizing plaques. Excessive free radicals, such as superoxide anion and hydroxyl radical, can attack biomembranes, proteins, and nucleic acids, resulting in cellular and tissue damage. Studies have found hirudin can significantly reduce the level of malondialdehyde (MDA), a lipid peroxidation product, in vivo. This reduction suggests that hirudin can inhibit lipid peroxidation and decrease free radical-induced damage to biomembranes. In addition, hirudin enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). SOD catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide, while GSH-Px reduces hydrogen peroxide to water. Together, these enzymes enhance the body's antioxidant capacity, reduce free radical accumulation, and mitigate oxidative stress-induced injury to vascular endothelial cells and plaques. Oxidative stress is known to activate intracellular signaling pathways, such as the MAPK pathway, leading to increased expression of inflammatory cytokines and cellular apoptosis. Studies have shown that hirudin can inhibit the phosphorylation of key proteins in the MAPK signaling pathway, thus blocking its activation, decreasing the expression of inflammatory cytokines, and reducing apoptosis, thereby stabilizing atherosclerotic plaques.

4.4 Anti-Platelet Aggregation Effects

Platelet activation and aggregation are critical in thrombus formation following atherosclerotic plaque rupture. Hirudin significantly reduces the risk of thrombosis by inhibiting platelet activation and aggregation, thus playing a vital protective role in plaque stability. Thrombin is a potent inducer of platelet activation and aggregation. By specifically binding with thrombin and forming a stable complex, hirudin inhibits its activity, thereby attenuating its ability to induce platelet activation and aggregation. Studies have shown that in in vitro experiments, the addition of hirudin markedly reduced thrombin-induced platelet aggregation rates. Hirudin can also directly act on platelets, inhibiting their surface receptors and signaling pathways. The glycoprotein IIb/IIIa (GPIIb/IIIa) receptor on the platelet surface plays a key role in the aggregation process; hirudin is able to inhibit the activation of this receptor, thereby preventing the binding of fibrinogen to platelets and ultimately suppressing aggregation. In addition, hirudin can also inhibit the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway during platelet activation, thereby reducing platelet adhesion and aggregation. In animal studies after intervention with hirudin, the expression of platelet activation markers such as membrane glycoprotein CD62P is decreased, indicating that it can effectively inhibit platelet activation, lower the risk of thrombosis, and maintain the stability of atherosclerotic plaques.

5. Prospects

As a naturally derived polypeptide, hirudin has demonstrated multidimensional positive effects on atherosclerotic plaque stability; however, its clinical utility requires further validation. Currently, hirudin shows some efficacy in improving disease conditions, but large-scale, multi-center, and long-term clinical trials are required to clarify its therapeutic efficacy, safety, optimal dosage, and treatment duration for different types of atherosclerotic diseases, in order to provide a basis for precision therapy. Mechanistically, hirudin influences plaque stability via multiple pathways; however, the synergistic interactions of these mechanisms and their dominant role at different pathological stages remain incompletely understood and require in-depth investigation using advanced molecular biology techniques. Moreover, the development of hirudin dosage forms is crucial; creating more stable and efficient delivery systems may improve its bioavailability and enhance therapeutic effects. Atherosclerosis is a multifactorial disease, and the therapeutic effects of hirudin may need to be combined with other drugs or interventions. Future research should explore synergistic mechanisms with other anti-atherosclerotic agents and optimize comprehensive treatment protocols. In summary, hirudin holds great promise in atherosclerosis research. Future directions should focus on mechanism elucidation, clinical validation, drug development, and combination therapy strategies to provide more effective approaches for the prevention and treatment of related diseases and improve patient prognosis.

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